In the Title:

Please replace the Title with the following Title:

P-HYDE SEQUENCES IN THE RAT

In the Specification:

Please replace the paragraph beginning on page 1, line 5, with the following rewritten paragraph:

--CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part application of U.S. Patent Application No. 09/449, 817, filed November 26, 1999, which is a continuation-in-part of U. S. Serial No. 09/302,457, filed April 29, 1999, the contents of which are hereby incorporated by reference—

Please replace the paragraph beginning on page 5, line 28 with the following rewritten paragraph:

--This invention provides a method of inhibiting the growth [[a]] of prostate cancer cells, comprising: 1) obtaining a sample of prostate cells from a subject; 2) contacting the cells with a replication deficient adenovirus type 5 expression vector which comprises an adenovirus genome having a deletion in the E1 and E3 regions of the genome and an insertion within the regions of a p-Hyde cDNA under the control of a Rous Sarcoma virus promoter; and 3) introducing the cells into the subject, thereby inhibiting the growth of the cancer cells.-

Please replace the paragraph beginning at page 7, line 31 with the following rewritten paragraph:

Fig. 1. Schematic presentation of AdRSVpHyde structure. The 2664 bp inserted fragment contains a 1467 bp full-length pHyde cDNA gene (SEQ ID NO:[[1]]3) and 1166 bp 3' untranslated downstream region. The complete sequence of AdRSVpHyde is set forth in Figure 10[[,]]. Specifically, the nucleic acid sequence of region A in Figure 1 is set forth in Figure 10 Region A (SEQ ID NO: 5) and the nucleic acid sequence of region B in Figure 1 is set forth in Figure 10 at Region B (SEQ ID NO: 6).

Please replace the paragraph beginning at page 9, line 25 with the following rewritten paragraph:

Figure 10. The complete sequence of AdRSVpHyde. Region A of AdRSVpHyde (SEQ ID NO: 5). Region B of AdRSVpHyde (SEQ ID NO: 6).

Please replace the paragraph beginning on page 15, line 13 with the following rewritten paragraph:

--A "nucleic acid" refers to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules") in either single stranded form, or a double-stranded helix. Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not lim it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear or circular DNA molecules (*e.g.*, restriction fragments), plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5[[?]]' to 3[[?]]' direction along the nontranscribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA). A "recombinant DNA" is a DNA that has undergone a molecular biological manipulation.-

Please replace the paragraph beginning on page 20, line 26 with the following rewritten paragraph:

--Adenovirus is easy to grow and manipulate and exhibits broad host range in vitro and in vivo. This group of viruses can be obtained in high titers, e.g., $10[[<9>]]^{9}$ –$10[[<11>]]^{11}$ plaque-forming unit (PFU)/ml, and they are highly infective. The life cycle of adenovirus does not require integration into the host cell genome. The foreign genes delivered by adenovirus vectors are episomal, and therefore, have low genotoxicity to host cells. No side effects have been reported in studies of vaccination with wild-type adenovirus (Couch et al., 1963; Top et al., 1971), demonstrating their safety and therapeutic potential as in vivo gene transfer vectors.-

Please replace the paragraph beginning on page 26, line 8 with the following rewritten paragraph:

-- High stringent hybridization conditions are selected at about 5[[?]] ºC lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typically, stringent conditions will be those in which the salt concentration is at least about 0.02 molar at pH 7 and the temperature is at least about 60? ºC. As other factors may significantly affect the stringency of hybridization, including, among others, base composition and size of the complementary strands, the presence of organic solvents, ie. salt or formamide concentration, and the extent of base mismatching, the combination of parameters is more important than the absolute measure of any one. For Example high stringency may be attained for example by overnight hybridization at about 68 ºC in a 6x SSC solution, washing at room temperature with 6x SSC solution, followed by washing at about 68 ºC in a 6x SSC in a 0.6x SSX solution.-

Please replace the paragraph beginning on page 26, line 21 with the following rewritten paragraph:

-- Hybridization with moderate stringency may be attained for example by: 1) filter pre-hybridizing and hybridizing with a solution of 3x sodium chloride, sodium citrate (SSC), 50% formamide, 0.1M Tris buffer at Ph 7.5, 5x Denhardt's solution; 2.) pre-hybridization at 37[[?]] ºC for 4 hours; 3) hybridization at 37[[?]] ºC with amount of labelled probe equal to 3,000,000 cpm total for 16 hours; 4) wash in 2x SSC and 0.1% SDS solution; 5) wash 4x for 1 minute each at room temperature at 4x at 60? ºC for 30 minutes each; and 6) dry and expose to film. -

Please replace the paragraph beginning on page 41, line 5 with the following rewritten paragraph:

-- The following non-classical amino acids may be incorporated in the peptide in order to introduce particular conformational motifs: 1,2,3,4-tetrahydroisoquinoline-3-carboxylate (Kazmierski et al., 1991, J. Am. Chem. Soc. 113:2275-2283); (2S,3S)-methyl-phenylalanine, (2S,3R)-methyl-phenylalanine, (2R,3S)-methyl-phenylalanine and (2R,3R)-methyl-phenylalanine (Kazmierski and Hruby, 1991, Tetrahedron Lett.); 2-aminotetrahydronaphthalene-2-carboxylic acid (Landis, 1989, Ph.D. Thesis, University of Arizona); hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (Miyake et al., 1989, J. Takeda Res. Labs. 43:53-76); [[?]]α-carboline (D and L) (Kazmierski, 1988, Ph.D. Thesis, University of Arizona); HIC (histidine isoquinoline carboxylic acid) (Zechel et al., 1991, Int. J. Pep. Protein Res. 43); and HIC (histidine cyclic urea) (Dharanipragada).-

Please replace the paragraph beginning on page 72, line 29 with the following rewritten paragraph:

-- In another embodiment one may irradiate the localized tumor site with DNA damaging radiation such as X-rays, UV-light, gamma-rays or even microwaves. Alternatively, the tumor cells may be contacted with the DNA damaging agent by administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a DNA damaging compound such as, adriamycin, 5-fluorouracil, etoposide, camptothecin, actinomycin-D, mitomycin C, or more preferably, cisplatin. The DNA damaging agent may be prepared and used as a combined therapeutic composition, or kit, by combining it with a p-Hyde expression construct, as described above. Agents that directly cross-link nucleic acids, specifically DNA, are envisaged and are shown herein, to eventuate DNA damage leading to a synergistic antineoplastic combination. Agents such as cisplatin, and other DNA alkylating may be used. Cisplatin has been widely used to treat cancer, with efficacious doses used in clinical applications of 20 mg/ m^2 [[<2 >]] for 5 days every three weeks for a total of three courses. Cisplatin is not absorbed orally and must therefore be delivered via injection intravenously, subcutaneously, intratumorally or intraperitoneally. Agents that damage DNA also include compounds that interfere with DNA replication, mitosis and chromosomal segregation. Such chemotherapeutic compounds include adriamycin, also known as doxorubicin, etoposide, verapamil, podophyllotoxin, and the like. Widely used in a clinical setting for the treatment of neoplasms, these compounds

are administered through bolus injections intravenously at doses ranging from 25-75 mg/ m^2[[<2 >]] at 21 day intervals for adriamycin, to 35-50 mg/m^2[[<2 >]] for etoposide intravenously or double the intravenous dose orally.-

Please replace the paragraph beginning on page 77, line 32 with the following rewritten paragraph:

-- **Characterization of cDNA**: *Sequencing- p-Hyde* cDNA was originally obtained as a [[?]] λ ZAP Uni XR clone, and was further subcloned into pBluescript SK^- vector through *in vivo* excision protocol as described (Stratagene, La Jolla, California). This double-stranded cDNA was further subjected for Dye Terminator Cycle Sequencing (Perkin Elmer, Foster City, California) using ABI 377 automatic DNA sequencer Version 3.0. The open reading frame of *p-Hyde* cDNA was determined using the DNA Strider program (Pasteur Institute, Paris).

Please replace the paragraph beginning on page 78, line 26 with the following rewritten paragraph:

-- ***Subcloning of p-Hyde into pcDNA3.1 (-)***: cDNA insert was released from the pBluescript SK^- vector through double digests by KpnI and SacI (SK fragment). This fragment represents the intact *p-Hyde* sequence and was then ligated into dephosphorylated KpnI-SacI double digests of mammalian shuttle vector pcDNA 3.1 (-) (Invitrogen). Ligation mix was used to transfect competent DH5[[?]]α, selected for ampicillin resistance followed by plasmid preparation using standard cesium chloride density gradient centrifugation. The new construct of *p-Hyde* was then used to transfect AT3 rat prostatic cancer subline by using lipofectamine (Gibco/BRL) followed by G418 selection (Rinaldy et al., 1988). Eight clones were obtained and two of them, AT3-H1 and AT3-H2, were used to assess the function of *p-Hyde* in its association with apoptosis. In addition, AT3 cell line was also transfected with pcDNA3.1(-) vector only and its stable transfected cel l line, designated as AT3-pc was used

as negative control relative to stable transfectant of AT3-H1 and AT3-H2 for the functional assessment of the *p-Hyde*. -

Please replace the paragraph beginning on page 79, line 25 with the following rewritten paragraph:

-- **Uridine Phosphorylase Assay**: Cell extract will be prepared from the cell pellet [[B]]before and after the induction with 1 mM 5-dFUrd for 24 hours, the corresponding cell extract will be prepared in 50 mM Potassium Phosphate buffer pH 7.4 through sonication followed by dialysis against the reaction buffer (50 mM potassium phosphate, pH 7.4). The amount of protein will be determined by using standard Lowry or Biorad assay. The same amount of protein from all cell lines will be assayed for UP activity in 50 mM potassium phosphate buffer (pH 7.4) containing 10 mM uridine or thymidine as substrate. After 30 min incubation at 37° C, the reaction will be terminated by adding methanol followed by centrifugation. An aliquot of the supernatant will be run on HPLC column (6 x 200 mm) of ERC-ODS-1171 (ERMA CR, Inc). The amount of reaction product Uracil or Thymidine can be measured with UV detector at 265 nm compared to the standard. As negative control, the similar reaction mixture will be boiled before the incubation. -

Please replace the paragraph beginning on page 80, line 8 with the following rewritten paragraph:

--**Construction of cDNA libraries:** In the first stage, cDNA libraries derived from AT-1 and MAT-LyLu cell lines were generated using Uni ZAP XR vector based on the protocol of Stratagene. The independent clones obtained were 1.9 and 3.4 million clones for MAT-LyLu and AT-1, respectively. These unamplified libraries were subjected to PCR amplification of the cDNA insert population. Reverse primers (RP) and forward primers (FP), downstream and upstream of XhoI and Eco RI cloning site, were used to amplify the cDNA insert population. The distance between both primers in [[?]] λ Uni ZAP or pBluescript was 228 bases.-

Please replace the paragraph beginning on page 80, line 16 with the following rewritten paragraph:

-- **Design of competition probes.** Two PCR probes were amplified: radiolabeled MAT-LyLu cDNA population probe and the non-radiolabeled AT-1 cDNA population probe (the cold competitor). The radiolabeled MAT-LyLu PCR product was enriched using S400 Sephacryl spin column. The majority of the unincorporated ^{32}P-dCTP, primer dimer, and 228 bp of PCR product resulting from the amplification of [[?]]λ DNA without insert, was separated from the cDNAs. The purified radiolabeled cDNAs were mixed with 30-fold excess of non-radiolabeled cold competitor AT-1 PCR products and used as a competition-probe to screen the MAT-LyLu cDNA library.-

Please replace the paragraph beginning on page 80, line 25 with the following rewritten paragraph:

--Two kinds of unexpected radiolabeled PCR products that may potentially interfere with the hybridization between the radiolabeled cDNA of the competition probe and the screened cDNA of the library were: 1) the non-exponential amplification of the cDNAs, and 2) 228 bp PCR product derived from the [[?]]λ DNA without cDNA insert. In order to reduce the possible cross-hybridization between these two unexpected PCR products with the vector of the screened library, excess amounts of HindIII-digested_DNA, PvuII-digested-pBluescript DNA, and the 228 bp PCR product of the pBluescript based on both primers were mixed with the competition probe. Preliminary assessment of this complete mixed competition probe indicated that the hybridization of the MAT-LyLu cDNA library with this probe was extremely weak; whereas the duplicate filter hybridized with the same probe, but without non-radiolabeled AT-1 PCR product, was extremely positive. This clearly indicate that the positive hybridization of the MAT-LyLu products was due to the radiolabeled MAT-LyLu cDNAs of the PCR products which was not competed by the AT-1 cDNAs.

Please replace the paragraph beginning at page 90, line 13 with the following rewritten paragraph:

Construction of AdRSVpHyde: A rat pHyde cDNA gene was isolated as described in U.S. Serial No: 09/302,457. After digestion with EcoRI, a 2.6 kb fragment which contains the 1467 bp full-length coding sequence of pHyde cDNA was subcloned under the control of a truncated RSV promoter (395 bp) into an E1/E3 deleted adenoviral shuttle vector. The resultant adenoviral shuttle vector was cotransfected into 293 cells with pJM17, an adenoviral type 5 genome plasmid, by calcium phosphate method. Individual plaques were screened for recombinant AdRSVpHyde by PCR using specific primers for both the RSV promoter and pHyde cDNA sequences. Single viral clones were propagated in 293 cells. The culture medium of the 293 cells showing the completed cytopathic effect (CPE) was collected, and the adenovirus was purified and concentrated by twice CsC12 gradient untracentrifugation. The viral titration and transduction were performed as previously described. The schematic diagram of AdRSVpHyde was illustrated in Fig. 1. The sequence of AdRSVpHyde is set forth in Figure 10 (SEQ ID NO: 5 and SEQ ID NO: 6).

Please replace the paragraph beginning on page 92, line 5 with the following rewritten paragraph:

-- ***DNA extraction and gel electrophoretic analysis of DNA fragmentation:*** Soluable DNA was extracted as described previously (in Oridate N, Lotan D, Xu X-C, Hong WK, and Lotan R. Differentiation induction of apoptosis by all-trans-retinoic acid and N-(4-hydroxyphenyl)retinamide in human head and neck squamous cell carcinoma cell lines. *Clin. Cancer Res.* 1996;2:855-863) Briefly, the cells floating in medium were collected 48 h post transduction by centrifugation. The pellet was resuspended in Tris-EDTA buffer (pH 8.0). The cells were lysed in 10 mM Tr-s-HCl (pH 8.0), 10 mM EDTA, and 0.5% Triton X-100 on ice for 15 min. The lysate was centrifuged at 12,000xg for 15 min to separate soluble (fragmented) DNA from pellet (intact genomic) DNA. Soluble DNA was treated with Rnase A (50 ug/ml) at 37_°C for 1 h, followed by treatment with proteinase K (100 ug/ml) in 0.5% SDS, at 50_°C for 2 h. The residual material was extracted with phenol/chloroform, precipitated in ethanol, electrophoresed on a 2% agarose gel.-